*Full Paper***Effects of Antiepileptics on Behavioral and Electroencephalographic Seizure Induced by Pentetrazol in Mice**Yusuke Watanabe¹, Kenshi Takechi¹, Akinori Fujiwara¹, and Chiaki Kamei^{1,*}¹*Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Tsushima-naka 1-1-1, Kita-ku, Okayama 700-8530, Japan**Received August 8, 2009; Accepted January 6, 2010*

Abstract. The present study was undertaken to investigate changes of the electroencephalogram (EEG) induced by pentetrazol (PTZ) in comparison with behavioral seizures in mice. Under pentobarbital anesthesia, mice were fixed to a stereotaxic apparatus, and electrodes were implanted into the frontal and occipital cortex. Behavioral and EEG changes were observed for 30 min following PTZ administration. After PTZ administration, mice showed myoclonic seizure (MCL) and clonic seizure (CL) in order. At the same time, spiking activity and spike-wave discharge in the cortex were observed. Phenobarbital, sodium valproate, diazepam, ethosuximide, and gabapentin caused a dose-dependent shortening of the duration of MCL and CL. In addition, they shortened the duration of spiking activity and spike-wave discharge dose-dependently. Moreover, phenytoin significantly inhibited the duration of spiking activity. It can be concluded that PTZ-induced spiking activity and spike-wave discharge serve as useful indices to assess the potential of antiepileptic activity in absence and MCLs in humans. Moreover, it is supposed that employing an index of EEG activity in addition to that of behavioral activity is desirable for objectivity.

Keywords: pentetrazol, myoclonic seizure, clonic seizure, electroencephalogram (EEG)

Introduction

Pentetrazol (PTZ)-induced seizure is widely used as a human absence epilepsy model to evaluate anticonvulsant efficacy (1, 2). In experimental mice, myoclonic seizure (MCL), including twitches and straub tail reaction, clonic seizure (CL), tonic flexion (TF), and tonic extension (TE), were observed after PTZ administration (3 – 5). Among these behaviors, MCL and CL observed in mice are mainly used as a model of human absence seizure in the field of behavioral pharmacology (6, 7). On the other hand, the nucleus raphe magnus and the pontine reticular formation play an important role in the expression of MCL and CL, respectively (8, 9). In many studies, MCL and CL are measured with the naked eye. Although such an observation method is often used in the field of behavioral pharmacology, its main disadvantage is the lack of objectivity. Moreover, it is thought that we cannot precisely evaluate drug effects on the central nervous

system with only observation of behavior.

It is well known that the changes of electroencephalogram (EEG) are observed in patients with generalized and partial seizures (10, 11), and EEG data are useful information in the diagnosis of epilepsy (12). In human absence epilepsy, a bilaterally synchronous 3-Hz spike and wave complex is mainly observed on the EEG (13). On the other hand, PTZ-induced seizure shows fast trains of spike discharges and subsequently a slow wave appears on the EEG (14, 15). We have reported that EEG changes induced by maximal electroshock were a useful index to assess the potential of antiepileptic activity (16); however, there is little information about the detailed EEG changes induced by PTZ administration in animals, except for a few papers. It is presumed that the measurement of EEG changes will provide new information that cannot be obtained with only behavior observation; therefore, we measured EEG changes as well as seizure symptoms induced by PTZ in detail. The effects of certain antiepileptics on both EEG changes and behavioral seizures were also studied.

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Materials and Methods

Experimental animals

Male ICR mice, 4-week-old (body weight, 20 – 24 g) were purchased from Japan SLC (Shizuoka). Animals were maintained in an air-conditioned room with controlled temperature ($24 \pm 2^\circ\text{C}$) and humidity ($55 \pm 15\%$). They were housed in plastic cages with sawdust under a light–dark cycle (lights on from 07:00 to 19:00). The mice were allowed free access to food and water, except during experiments. Twenty mice were used for correlation study. Eight mice were used for each dose of the drugs in the study for an assessment of drug efficacy. Therefore, 84 mice were used in this study. One animal was used for only one dosage of a drug. All procedures involving animals were carried out in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Surgery

The surgery was performed similarly to the method described previously (17 – 19). Under pentobarbital anesthesia (60 mg/kg, i.p., Nembutal®; Abbot Laboratories, North Chicago, IL, USA), the mice were fixed to a stereotaxic apparatus (SR-5, Narishige; Tokyo), and monopolar screw electrodes were implanted in the frontal pole cortex (FP; 3.0 mm anterior to the bregma, 1.0 mm lateral to the midline) and the right or left occipital cortex (ROCOR or LOCOR, 4.3 mm posterior to the bregma, 2.4 mm lateral to the midline) according to the atlas of Franklin and Paxinos (20). The electrodes were connected to a miniature receptacle, which was embedded in the skull with dental cement. At least 1 week was allowed for recovery from the surgery.

Experimental procedures

Study for an assessment of drug efficacy: The animals were placed in a plastic cage ($32 \times 18 \times 24$ cm) and convulsion was induced by subcutaneous injection of PTZ. After PTZ (75 mg/kg) administration, MCL, including fore- and hind-limb twitches and straub tail reaction, CL, TF, and TE, were observed. Animals were observed for 30 min after PTZ administration. The durations of MCL and CL were used as criteria of anticonvulsant efficacy. EEG was recorded bipolarly (FP-LOCOR and FP-ROCOR) with an electroencephalograph (EEG-7414; Nihon Kohden, Tokyo) and analyzed with a multi-purpose bio-information analysis program (BIMUTASII; Kissei Comtec, Nagano). EEG changes were divided into the following 2 stages: spiking activity and spike-wave discharge. Spiking activity has the following features: appearance at intervals of 5 s or less, duration of 20 – 80 ms, and amplitude of more than $100 \mu\text{V}$. Spike-wave

discharge has the following features: frequency of 4 – 10 Hz and amplitude of more than $100 \mu\text{V}$.

Correlation analysis: Twenty animals were injected with PTZ (75 mg/kg) subcutaneously. Animals were observed for 30 min after PTZ administration. The duration of MCL, CL, spiking activity, and spike-wave discharge were measured. The total duration of these criteria for 30 min was summed, and then the regression line was derived by the least-squares method. The strength of the bivariate linear relationship, that is, between the duration of MCL and spiking activity or the duration of CL and spike-wave discharge, was examined using a correlation coefficient calculated by the method of Pearson.

Drugs

PTZ (Sigma, St. Louis, MO, USA) was dissolved in saline and administered at a volume of 5 ml/kg of body weight. Phenytoin (Wako, Osaka), phenobarbital sodium (Wako), sodium valproate (Wako), carbamazepine (Sigma), diazepam (Sigma), ethosuximide (Sigma), topiramate (Sigma), and gabapentin (Sigma) were used as the anticonvulsants. These drugs were suspended in 0.5% carboxymethyl cellulose (CMC) solution and orally administered 1 h before PTZ administration at a volume of 5 ml/kg of body weight. Control groups or animals used for correlation analysis received 0.5% CMC solution.

Statistical analyses

All data are expressed as the means $\pm$ S.E.M. ANOVA and Dunnett's tests were used for statistical analysis of MCL, CL, spiking activity, and spike-wave discharge duration. Correlations were examined according to the method of Pearson. A *P*-value less than 0.05 was considered significant.

Results

Effects of antiepileptics on the duration of MCL

No marked behavioral effects were observed with the antiepileptics used in this study, except for phenobarbital and diazepam. Phenobarbital at doses of 20 and 50 mg/kg and diazepam at doses of 2 and 5 mg/kg resulted in a slight degree of sedation or muscular relaxation in some mice. The results are shown in Fig. 1. The duration of MCL induced by PTZ was dose-dependently shortened by phenobarbital, sodium valproate, diazepam, ethosuximide, and gabapentin. Significant differences were observed with phenobarbital at a dose of 50 mg/kg [$F(3,28) = 6.55$, $P < 0.01$], sodium valproate at doses of 200 and 500 mg/kg [$F(3,8) = 23.04$, $P < 0.01$], diazepam at doses of 2 and 5 mg/kg [$F(3,28) = 5.80$, $P < 0.01$], ethosuximide at doses of 500 and 1000 mg/kg [$F(3,28) = 14.98$,

Fig. 1. Effects of antiepileptics on the duration of MCL. Each column and vertical bar represents the mean $\pm$ S.E.M. ($n = 8$). * $P < 0.05$, ** $P < 0.01$: significantly different from the control group. MCL, myoclonic seizure.

Fig. 2. Effects of antiepileptics on the duration of CL. Each column and vertical bar represents the mean $\pm$ S.E.M. ($n = 8$). * $P < 0.05$, ** $P < 0.01$: significantly different from the control group. CL, clonic seizure.

$P < 0.01$], and gabapentin at doses of 100 and 200 mg/kg [$F(3,28) = 11.94$, $P < 0.01$]. On the other hand, carbamazepine showed significant prolongation at a dose of 50 mg/kg [$F(3,28) = 3.24$, $P < 0.05$]. Phenytoin [$F(3,28) = 0.37$, $P = 0.777$] and topiramate [$F(3,28) = 0.64$, $P = 0.600$] showed no significant effects on MCL duration.

Effects of antiepileptics on the duration of CL

The results are shown in Fig. 2. The duration of CL induced by PTZ was dose-dependently shortened by

phenobarbital, sodium valproate, diazepam, ethosuximide, and gabapentin. Significant differences were observed with phenobarbital at doses of 20 and 50 mg/kg [$F(3,28) = 9.35$, $P < 0.01$]; sodium valproate at doses of 200 and 500 mg/kg [$F(3,28) = 6.22$, $P < 0.01$]; diazepam at doses of 1, 2, and 5 mg/kg [$F(3,28) = 13.65$, $P < 0.01$]; ethosuximide at doses of 500 and 1000 mg/kg [$F(3,28) = 7.64$, $P < 0.01$]; and gabapentin at doses of 100 and 200 mg/kg [$F(3,28) = 5.16$, $P < 0.01$]. On the other hand, phenytoin and carbamazepine prolonged CL duration

Fig. 3. Representative example of electroencephalographic changes induced by pentetrazol. A) Representative EEG examples of the control group. B) Enlarged EEG of the control group. SA, spiking activity; SWD, spike-wave discharge; FP, frontal pole cortex; LOCOR, left occipital cortex; ROCOR, right occipital cortex.

dose-dependently, and significant differences were observed with phenytoin [$F(3,28) = 2.97$, $P < 0.05$], and carbamazepine [$F(3,28) = 5.70$, $P < 0.01$] at a dose of 50 mg/kg, respectively. Although topiramate [$F(3,28) = 1.48$, $P = 0.263$] showed a tendency to prolong CL duration, significant difference was not observed.

Behavioral and EEG seizures induced by PTZ

Figure 3A shows representative EEG seizures induced by subcutaneous injection of PTZ at a dose of 75 mg/kg. At 0–5 min after PTZ administration, spiking activity with a duration of 20–80 ms was observed in parallel with MCL, including fore- and hind-limb twitches and tail movements. Subsequently, spike-wave discharge with a frequency of 4–10 Hz was observed in parallel with CL at 5–10 min after PTZ administration. Enlarged EEGs were shown in Fig. 3B, which was obtained from the same mouse as Fig. 3A. Overt spikes or spike and wave complex were observed on enlarged control EEGs.

Figure 4 shows the relationship between the duration of behavioral seizure and EEG seizure after PTZ administration. Spike activity and spike-wave discharge were observed along with MCL and CL, respectively. The regression line of the duration of MCL seizure and spiking activity was $Y = 1.686 X + 744.7$ (A). The regression line of the duration of CL seizure and spike-wave discharge was $Y = 0.617 X + 22.26$ (B). To examine the strength of these linear relationships, we calculated the correlation coefficient using the method of Pearson. As a result, there was a significant correlation between MCL seizure and spiking-activity duration ($r = 0.676$, $P < 0.01$) and CL duration and spike-wave discharge duration ($r = 0.686$, $P < 0.01$).

Effects of antiepileptics on the duration of spiking activity

Figure 5 shows representative spiking activity when animals were administered phenytoin at a dose of 50 mg/

Fig. 4. Relationship between the durations of seizure and EEG induced by pentetrazol. A: SA duration and MCL duration. B: SWD duration and CL duration. MCL, myoclonic seizure; CL, clonic seizure; SA, spiking activity; SWD, spike-wave discharge.

Fig. 5. Representative example of effects of phenytoin (50 mg/kg, p.o.), topiramate (100 mg/kg, p.o.), and gabapentin (200 mg/kg, p.o.) on spiking activity induced by pentetrazol. SA, spiking activity; FP, frontal pole cortex; LOCOR, left occipital cortex; ROCOR, right occipital cortex.

kg, topiramate at a dose of 100 mg/kg, and gabapentin at a dose of 200 mg/kg. Phenytoin and gabapentin showed a tendency to shorten spiking-activity duration. On the other hand, the spiking-activity duration showed almost no change by administration of topiramate. Figure 6 shows the effects of antiepileptics on the duration of spiking activity. The duration of spiking activity induced by PTZ was dose-dependently shortened by phenytoin, phenobarbital, sodium valproate, diazepam, ethosuximide, and gabapentin. Significant difference were observed with phenytoin at doses of 20 and 50 mg/kg [$F(3,28) = 4.87$, $P < 0.01$]; phenobarbital at doses of 20 and 50 mg/kg [$F(3,28) = 11.99$, $P < 0.01$]; sodium valproate at doses of 200 and 500 mg/kg [$F(3,28) = 10.24$, $P < 0.01$]; diazepam at doses of 1, 2, and 5 mg/kg [$F(3,28) = 52.69$, $P < 0.01$]; ethosuximide at doses of 200, 500, and 1000 mg/kg [$F(3,28) = 12.69$, $P < 0.01$]; and gabapentin at doses of 50, 100, and 200 mg/kg [$F(3,28) = 8.78$, $P < 0.01$]. On the other hand, carbamazepine [$F(3,28) = 0.23$, $P = 0.875$] and topiramate [$F(3,28) = 0.06$, $P = 0.981$] caused no significant effects even at doses of 50 and 100 mg/kg, respectively.

Effects of antiepileptics on the duration of spike-wave discharge

Figure 7 shows a representative spike-wave discharge when animals were administered phenytoin at a dose of 50 mg/kg, topiramate at a dose of 100 mg/kg, and gabapentin at a dose of 200 mg/kg. Gabapentin decreased the duration of spike-wave discharge; however, phenytoin and topiramate caused no marked changes in the duration of spike-wave discharge. As shown in Fig. 7, abnormal

Fig. 6. Effects of antiepileptics on the duration of spiking activity. Each column and vertical bar represents the mean $\pm$ S.E.M. ($n = 8$). * $P < 0.05$, ** $P < 0.01$: significantly different from the control group. SA, spiking activity.

Fig. 7. Representative example of effects of phenytoin (50 mg/kg, p.o.), topiramate (100 mg/kg, p.o.), and gabapentin (200 mg/kg, p.o.) on SWD induced by pentetrazol. SWD, spike-wave discharge; FP, frontal pole cortex; LOCOR, left occipital cortex; ROCOR, right occipital cortex.

EEG pattern was observed before and after appearance of spike-wave discharge. Those wave forms are unsuitable according to the criteria for spike-wave discharge as shown in the experimental procedure. Figure 8 shows the effects of antiepileptics on the duration of spike-wave discharge. The duration of spike-wave discharge induced by PTZ was dose-dependently shortened by phenobarbital, sodium valproate, diazepam, ethosuximide, and gabapentin. Significant differences were observed with phenobarbital at doses of 20 and 50 mg/kg [$F(3,28) = 3.00$, $P < 0.05$]; sodium valproate at doses of 200 and 500 mg/kg [$F(3,28) = 3.41$, $P < 0.05$]; diazepam at doses of 1, 2, and 5 mg/kg [$F(3,28) = 6.73$, $P < 0.01$]; ethosuximide at doses of 200, 500, and 1000 mg/kg [$F(3,28) = 3.43$, $P < 0.05$]; and gabapentin at doses of 50, 100, and 200 mg/kg [$F(3,28) = 3.87$, $P < 0.05$]. On the other hand, phenytoin [$F(3,28) = 0.07$, $P = 0.976$], carbamazepine [$F(3,28) = 0.38$, $P = 0.777$] and topiramate [$F(3,28) = 0.06$, $P = 0.980$] caused no significant effects even at doses of 50, 50, and 100 mg/kg, respectively.

Discussion

In the present study, it was confirmed that MCL and CL occurred by PTZ administration. Phenobarbital, sodium valproate, diazepam, ethosuximide, and gabapentin showed dose-dependent inhibition of both MCL and CL. On the other hand, phenytoin, carbamazepine, and topiramate showed no effect on either MCL or CL. These findings were almost identical to those of the previous reports (21–24). In this study, it was also found that

Fig. 8. Effects of antiepileptics on the duration of SWD. Each column and vertical bar represents the mean $\pm$ S.E.M. ($n = 8$). * $P < 0.05$, ** $P < 0.01$: significantly different from the control group. SWD, spike-wave discharge.

spiking activity and spike-wave discharge were observed in correlation with MCL and CL, respectively, after PTZ administration. It was reported that there is a close relationship between convulsive behavior and EEG seizures in epileptic patients (12). In addition, it is well known that EEG spike indicates the action potential of stimulated nerve cells, and excitation of the brain was observed in the area showing the EEG spike. Excitation of the brain resulted in convulsive behavior; that is, EEG findings such as spiking activity or spike-wave discharge were generated before convulsive behavior. Therefore, EEG seizures (spiking activity and spike-wave discharge), as well as behavioral seizures (MCL and CL), were used as an index for estimating the effects of certain antiepileptics on PTZ-induced seizures. As a result, phenobarbital, sodium valproate, diazepam, ethosuximide, and gabapentin caused a dose-dependent and significant inhibition of the durations of spiking activity and spike-wave discharge. Kostadinova et al. (25) and Marescaux et al. (26) also reported that phenobarbital, sodium valproate, diazepam, and ethosuximide showed inhibitory effects on PTZ-induced EEG discharge in rats. Therefore, EEG seizures induced by PTZ are a useful index for estimating anticonvulsant effects.

Kubová et al. (27) found that benzodiazepines associated with the GABAergic system inhibited spiking activity induced by cortical stimulation in rats. It is well recognized that sodium valproate and ethosuximide reduce T-type Ca^{2+} currents (28). In addition, van de Bovenkamp-Janssen et al. (29) suggested that the activation of low voltage-activated T-type Ca^{2+} channels is involved in the appearance of spike-wave discharges. Therefore, it is reasonable to assume that the inhibitory effects of diazepam, sodium valproate, and ethosuximide on the spiking activity and spike-wave discharge are produced by regulation of the GABAergic system or T-type Ca^{2+} currents. Gabapentin also inhibited PTZ-induced EEG seizures. It is known that gabapentin alters the metabolism or concentrations of glutamate, glutamine, or GABA in brain tissues and also interacts with the $\alpha_2\delta$ -subunit of voltage-gated Ca^{2+} channels (30); therefore, there is a possibility that these mechanisms are correlated with the suppressive action of gabapentin on PTZ-induced EEG seizures.

PTZ-induced MCL and CL are widely used to identify effective drugs for human absence epilepsy. In this study, phenytoin, carbamazepine, and topiramate did not inhibit the duration of MCL and CL. In clinical trials, these three drugs showed no effects on the symptoms of absence epilepsy (31). Interestingly, spiking activity induced by PTZ was inhibited significantly by phenytoin at relatively small doses; however, MCL was not inhibited by phenytoin at the same dose levels in this study. Similar findings

were also reported that phenytoin inhibited spiking activity (focal seizure) induced by cephaloridine administration (32). Ebert et al. (33) reported that phenytoin inhibited the generation of focal seizure activity. In contrast, it is well recognized that phenytoin showed no effect on the spread of seizure activity. MCL and CL are continuous events, and it was thought that these behavioral seizures are closely related with the spread of seizure activity, that is, propagation. Therefore, phenytoin showed potent inhibition of spiking activity, although the drug showed no obvious effects on MCL. In the present study, high doses of phenytoin and carbamazepine significantly prolonged the duration of CL; however, no significant effect was observed with the duration of spike-wave discharge. Brevard et al. (34) suggested that portions of the thalamus, especially anterior thalamic nuclei, contribute to the initiation of generalized tonic-CLs and appear to be the gateway propagating the epileptiform activity to the cortex. They were also discussed that the piriform, entorhinal, and olfactory cortices may be involved in the expression or maintenance of seizures. From these findings, it is reasonable to presume that phenytoin and carbamazepine preferentially act on the anterior thalamic nuclei that function as the gateway to the cortex and thereby inhibited an aggravation of EEG seizures only.

We compared the effects of behavioral seizures and EEG seizures inhibited by various antiepileptics. This revealed that antiepileptics that are effective in the absence seizures, that is, phenobarbital, sodium valproate, diazepam, ethosuximide, and gabapentin, showed almost the equivalent or more potent effect on EEG seizures compared with behavioral seizures. These results suggested that EEG seizures as well as behavioral seizures are useful indices to estimate the antiepileptic potential for absence seizures in humans. In addition, they provide new information for us that spiking activity may be a useful index for evaluating the drugs, such as phenytoin, effective against focal seizures.

In conclusion, it can be said that the measurements of PTZ-induced spiking activity and spike-wave discharge serve as reliable indices to assess the potential of antiepileptic activity in absence and MCLs in humans. Moreover, it is speculated that employing an index of EEG activity in addition to that of behavioral activity is desirable for objectivity.

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